International School for Advanced Studies

Decision timing in the face of changing sensory input: Behavior and neural correlates

Thesis submitted for the degree of
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Candidate:
Adina Drumea

Supervisor:
Prof. Mathew E. Diamond

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Cognitive Neuroscience sector
SISSA, Via Bonomea 265, TRIESTE, ITALY

Abstract

Most real-life situations require organisms to extract information from incoming stimuli to predict future events and, from the prediction, to precisely time the appropriate motor act. In the present study we designed a new behavioral task that requires subjects (humans and rodents) to extract temporal information characterizing a continuous stream of sensory input and execute a precisely timed motor act. Furthermore, we recorded neurons in the premotor cortex of rats performing this task to investigate the involvement of this brain area in timing actions as a response to incoming stimulation.

In our experiment rats received vibrations on their whiskers and responded by withdrawing from the nose-poke hole, while humans received the stimuli on their fingertips and responded by pressing a button. The stimuli were formed by multiplying pink-noise velocity values by an envelope sine wave. Responses made around the peak of the envelope (40% of each cycle) were rewarded. The parameters of the envelope (frequency, amplitude and phase at stimulus onset) changed from trial to trial to ensure that subjects could not set an absolute amplitude threshold or use timing alone (e.g. "wait 1 second after stimulus onset") to solve the task.

Rats and humans learned to time their responses to the envelope peak at above-chance levels across different envelope parameters. Both rats and humans responded in later cycles in high frequency and low amplitude stimuli, suggesting that these stimuli were more difficult and thus required integration of more evidence to support the response. Furthermore, rats benefited from collecting more information about the stimulus, as shown by better-timed responses made in the second than in the first cycle of stimulation.

As expected, the activity of premotor cortex neurons was predictive of the imminence of the animal's action, in the time period preceding the withdrawal. Moreover, neurons carried information regarding the stimulus, with a large proportion coding for the overall stimulus amplitude. A small percentage of the recorded premotor cortex neurons also showed a correlation between firing rate and the stimulus amplitude at any given point in the trial.

The strategy rats were likely to use for solving the task emerging from these results was to understand the global amplitude of the trial and set an amplitude threshold against which to compare the perceived stimulus.

Interestingly, the activity of premotor cortex neurons at different moments in the trial was correlated with the time at which the rat withdrew, carrying information both regarding how much time the rat is willing to wait and how much time has passed since the stimulation started. We used an artificial neural network (ANN) implemented in MATLAB to predict withdrawal time from the firing rates at different time bins of all the neurons recorded simultaneously within a behavioral session, and found a good network performance in the time bins preceding the animal's action. Performance was better in incorrect trials, indicating that in some trials rats only engaged in timing, while in others they paid attention to the stimulus and did not keep track of time.

In summary, we designed a new behavioral paradigm to investigate how the brain times decisions in response to changing incoming sensory stimulation. Both rats and humans learned to align their responses to the peak of the envelope and chose to gather more stimulus information in trials characterized by low amplitude and high frequency. Finally, neurons in the premotor cortex of rats performing the task carried signals related to key aspects of the task: the time of withdrawal and stimulus properties.

Contents

1. Introduction

1.1. Designing a task to measure temporal signals within noise

In our experiment subjects received a stream of noisy sensory stimulation based on whose properties they were required to time a motor response. The stochastic nature of the stimulus meant that subjects had to accumulate information about the stimulus in time.

Previous research has been aimed at understanding how the brain accumulates evidence about a noisy stream of sensory input across time. For instance, in the random dot motion discrimination (RDMD) task, subjects have to judge the direction of movement of dots presented on a screen and the difficulty is varied by changing the percentage of coherently moving dots (Figure 1.1.A). Theoretical models, such as the drift diffusion model (Smith, 2000) consider that evidence supporting one hypothesis about the stimulus (e.g. its direction of motion) is accumulated in a decision variable that drifts in time towards a decision boundary (Figure 1.1.B). The decision is made once the boundary has been reached. Accumulation of evidence in time was evident from increased performance for longer stimulus duration. Moreover, when allowed to collect stimulus information at their own pace, subjects waited more time before responding and were less accurate lower coherence than in higher coherence trials. These effects have been revealed in human (Watamaniuk & Sekuler, 1992), primate (Roitman & Shadlen, 2002) and rodent studies (Douglas, Neve, Quittenbaum, Alam, & Prusky, 2006; Reinagel, Mankin, & Calhoun, 2012).

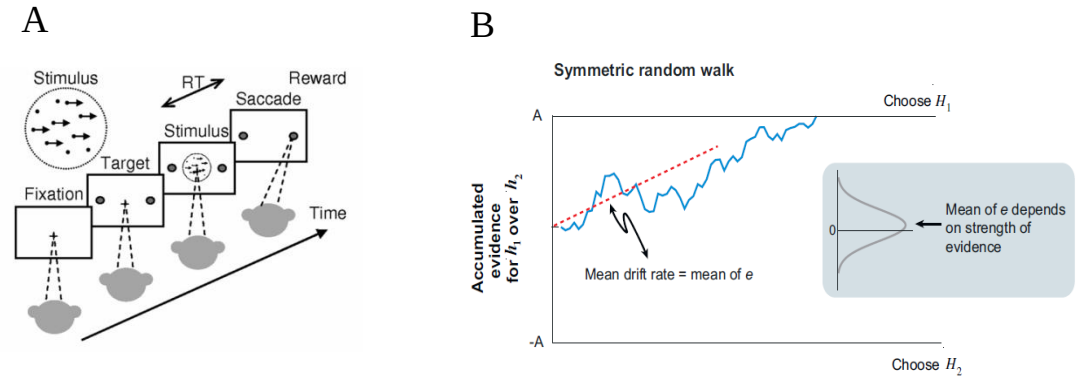

Figure 1.1: A. Random dot motion discrimination task (RDMD) requires subjects to judge the direction of motion of dots presented on the screen (Joshua I. Gold & Ding, 2013). B. Diffusion models, such as the random walk model could explain the behavior in tasks such as the RDMD. Evidence is accumulated at a rate depending on its strength (percentage of coherently moving dots), and the decision is made once the accumulated evidence reaches a threshold (Joshua I Gold & Shadlen, 2007).

While the use of noisy stimuli has been valuable in showing how the brain might accumulate evidence to reduce uncertainty, the decision to be made in such cases concerns the properties of the stimulus, not timing. In short, a random dot experiment can explore how the brain determines "what" but not how the brain determines "when". Our experiment originated with the idea of an underlying rhythm, albeit uncertain due to the noisy character. The rhythm allowed us to formulate a "when" question: when does the cyclical input reach a peak? A few similar experimental paradigms have been previously performed, only engaging human subjects. For example, participants were required to predict the time of occurrence of an auditory stimulus part of a rhythmic tone presentation (Arnal, Doelling, & Poeppel, 2014).

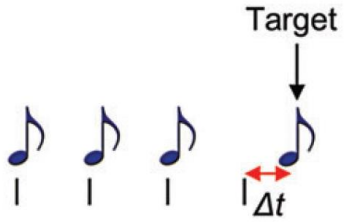

Figure 1.2: Example of a behavioral task that required humans to extract the temporal pattern of a sequence of tones and decide whether the last one was delayed with regard to the beat (Arnal et al., 2014).

The task we designed in the current study combines the accumulation of evidence in time to the extraction of temporal information about the stimulus and adds a new dimension: the preparation of a precisely timed motor act in response to the incoming stimulus.

1.2. The choice of stimuli

The stimuli used in our experiment were tactile vibrations delivered on the whiskers and fingertips of rats and humans respectively.

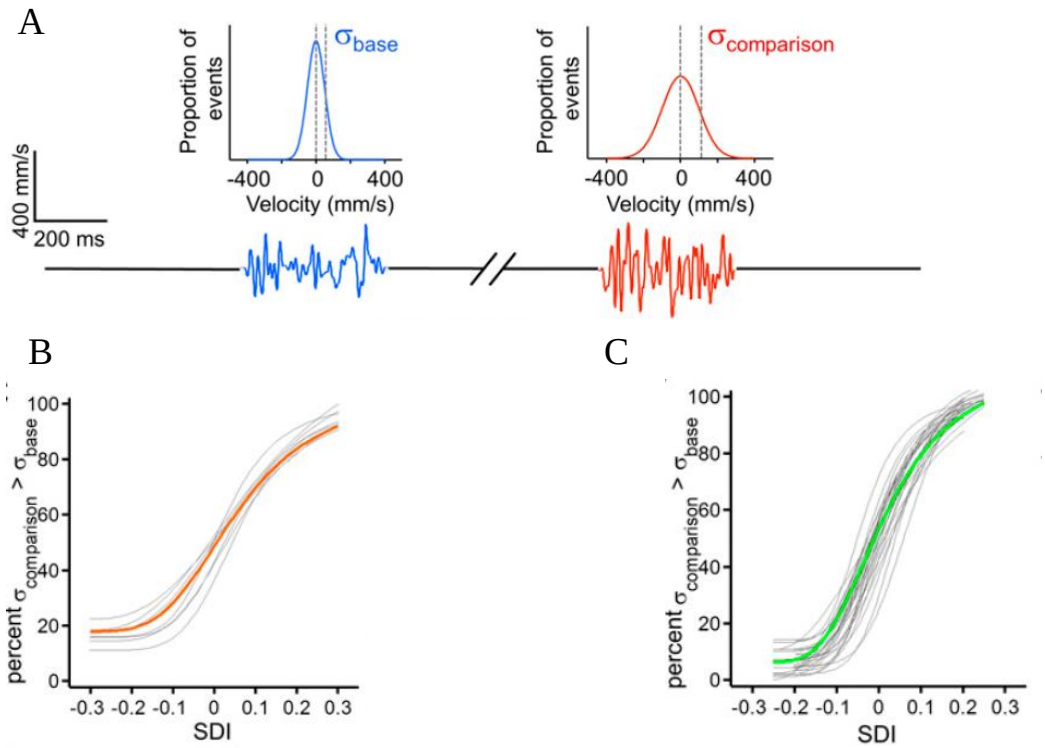

Figure 1.3: The vibrotactile working memory task requires subjects to perceive 2 consecutive vibrations and compare their strength, which is quantified by the standard deviation of the velocity values of each vibration (A). Psychometric curves of rats (B) and humans (C) performing this task (Fassihi, Akrami, Esmaeili, & Diamond, 2014).

Tactile stimuli are particularly useful when performing experiments on rodents, who have a highly developed sense of touch on which they rely for

survival. Moreover, the tactile perception of rodents and humans are highly comparable (Diamond, 2010). The behavior of humans performing the same tasks as rats exhibit very similar response patterns, suggesting that rodent studies are a useful tool for understanding how such processes take place in the human brain (Fassihi et al., 2014) (Figure 1.3. B, C).

Recent studies required rats to make decisions based on the properties of vibrations received passively through the whiskers (Adibi, Diamond, & Arabzadeh, 2012; Fassihi et al., 2014) (Figure 1.3. A). Such stimuli allow the experimenter to exercise a strict control over the parameters of the stimuli entering the sensory system. The use of vibrations is also ecologically relevant, as rodents are burrowing animals and could use information extracted from the earth vibrations to understand the size and direction of movement of an outside predator. Furthermore, the brain must decode the whisker vibrations resulting from rat's sweeping movements over textures in order to determine the coarseness of the texture (Lottem & Azouz, 2008, 2009).

1.3. The choice of the brain area to be examined

The brain area we focused on in the present study is the rat premotor cortex, considered to be the analogue of the primate premotor cortex (Condé, Maire-lepoivre, Audinat, & Crépel, 1995; Harry M. Sinnamon, 1984; James V. Corwin, 1998; Roger L. Reep, James V. Corwin, Atsutaka Hashimoto, 1984)

The primate premotor cortex has been traditionally viewed as dedicated to movement preparation (Crutcher & Alexander, 1990; Gentilucci et al., 1988; Riehle & Requin, 1989; Tanji, Taniguchi, & Saga, 1980), but new functions have emerged for motor and premotor brain areas, such as stimulus categorization (R Romo, Ruiz, Crespo, Zainos, & Merchant, 1993), evidence accumulation (Liu & Pleskac, 2011) and decision making (Hernández, Zainos, & Romo, 2002; Ranulfo Romo, Hernández, & Zainos, 2004; Ranulfo Romo, Hernández, Zainos, Lemus, & Brody, 2002).

The rat premotor cortex has been shown to contribute to decision making by conveying significant decision value and chosen value signals before and after a choice was made, respectively. Therefore, it might be part of the neural system where actions are selected and propagated to downstream motor

structures for execution (Sul, Jo, Lee, & Jung, 2011). Rat premotor cortex has also been shown to be crucial for orienting movements in a memory-guided task, as neurons respond selectively to contralateral or ipsilateral movements, and unilateral inactivation of this area impairs contralateral orienting movements (Erlich, Bialek, & Brody, 2011). Even if such tasks require subjects to accumulate evidence about the stimulus in time, rat premotor cortex has been shown to not be directly involved in evidence accumulation, but to represent the choice the rat would make based on the evidence accumulated thus far (Figure 1.4. A) (Hanks et al., 2015). Similarly, the mouse anterior lateral motor cortex (ALM), proposed to be the analogue of primate premotor cortex has been shown to be involved in planning licking. Neurons in this area show response preference for contralateral or ipsilateral licks, while ALM inactivation affects only contralateral licking movements (Guo et al., 2014; Li, Chen, Guo, Gerfen, & Svoboda, 2015).

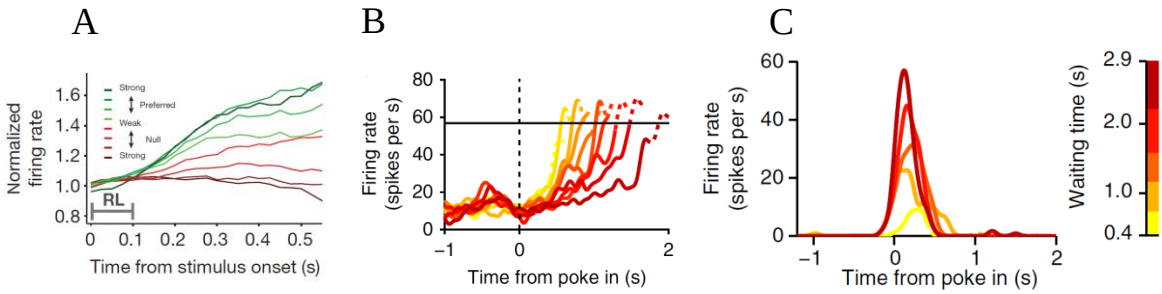

Figure 1.4: Functions of rat premotor cortex. A. The firing rate of a population of neurons in the premotor cortex ramped up at a rate proportional to the strength of the evidence in a task where rats had to decide which of two speakers delivered higher frequency auditory stimuli (Hanks et al., 2015). B. Example premotor cortex neuron ramping in the absence of any sensory stimuli. The rat responded when the neuron's firing rate reached a certain threshold. C. Example neuron with transient activation predicting how much the rat will wait in the nose poke in the absence of incoming stimulation (Murakami, Vicente, Costa, & Mainen, 2014).

Rat premotor cortex neurons have also been shown to be involved in keeping track of the time a rat is waiting for an incentive. In a task where rats could give up waiting for a large reward in favor of a small reward, a proportion of premotor cortex neurons transiently increased or decreased their activity at different time points in the trial, with their firing rate proportional to the time the rat waited (Figure 1.4.C), while other neurons gradually increased

or decreased their firing rates during the trial, reaching a firing threshold just before the animal's response (Figure 1.4.B). The authors suggested that the results could be explained by a neural integration process where the first kind of neurons are the input to the ramping integrator neurons that trigger an action when reaching a threshold (Murakami et al., 2014). Neurons in this area have been also linked with time measuring in a task in which different stimuli indicated the interval after which the reward would be available. As rats waited for the reward, the response patterns of motor cortex neurons could be described as ramps, peaks and dips and provided sufficient information to discriminate the delay duration (Matell, 2012).

1.4. Aim of the project

The current project is aimed at understanding the brain mechanisms responsible for deciding the precise timing of one's actions. Subjects in this study received noisy tactile vibrations based on which they had to plan their motor response. More precisely, the stimuli were modulated by a sinusoidal wave and a correct response (button press for humans or nose poke withdrawal for rats) was considered one at the peak of the stimulus. For each trial subjects had to extract the stimulus properties (sine amplitude and frequency) in order to predict when the next peak should occur and plan the timing of their motor response.

The brain area of interest was the rat premotor cortex. The premotor cortex has been previously shown to be involved in sensory decision making as well as time perception and action planning, making it a good candidate for supporting the timing of responses to incoming sensory stimuli.

2. Methods

2.1. Animal subjects

Five Wistar male rats (Harlan Laboratories, Italy) were used in this study. At the start of the experiments the animals were 6-8 weeks old. Rats were housed in pairs and maintained on a reversed 12/ 12 hours dark/ light cycle with *ad libitum* food, but water restricted during the experimental period.

Protocols were in accordance to international norms and were approved by the Italian Health Ministry and the Ethics Committee of the International School for Advanced Studies.

2.2. Apparatus

The apparatus consisted of a 25×25×38 cm (H×W×L) plexiglass chamber, custom-made by the SISSA Mechatronics Lab (Figure 2.1A). The front wall consisted of a central head hole opening through which rats could access the nose-poke hole. The nose-poke was a 0.7 cm diameter opening with an infrared sensor to detect the animal's presence (Figure 2.1B). On top of the opening a green LED light was placed to signal to the animal when it could initiate a new trial. Animals received vibrations on the vibrissae bilaterally by vibrating plates connected to motors (Brüel & Kjær Type 4809 shakers). Sticky tape was attached to the plates to ensure a better adherence of the whiskers. Rats received water reward through drinking spouts situated on one side. Animal licking was detected by infrared sensors in the drinking spout and triggered the activation of a syringe pump delivering the reward (Figure 2.1C). Two speakers mounted on the side walls of the apparatus delivered auditory cues. One cue was activated when the nose-poke sensor stopped detecting the animal's nose (response cue), informing the rat of the time at which a response was registered. The other cue signaled the reward delivery (reward cue), working as an auditory reinforcement for the rat.

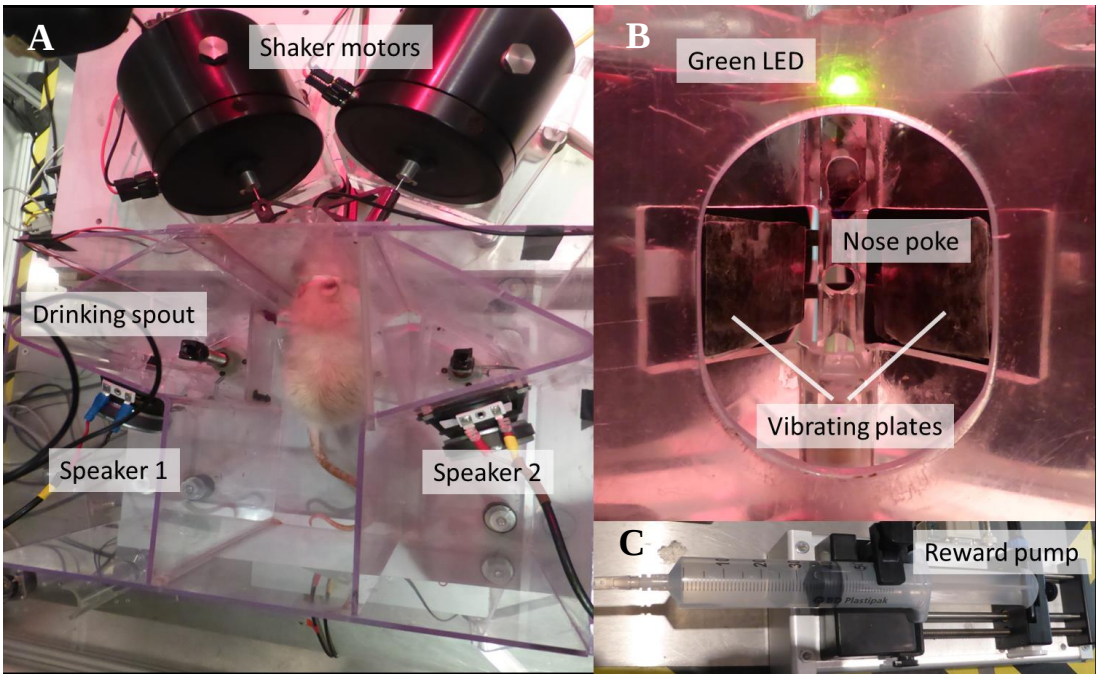

Figure 2.1: Photo of the experimental setup from above (A), point of view of the rat (B) and pump for delivery of water reward (C).

2.3. Stimuli

The stimuli were formed by multiplying a velocity noise vibration by an envelope sine wave (Figure 2.2A) and we refer to them as sine-modulated noisy vibrations. First a noisy vibration was obtained by choosing probe position values from a normal distribution with 0 mean and standard deviation of 1 mm, using a sampling rate of 10.000 values per second. The signal was then low pass filtered with a 150 Hz Gaussian filter and multiplied by a low frequency envelope with values between 0 and 1. The envelope wave parameters changed from trial to trial. The frequencies used were 0.7 and 1 Hz. The difference between the base and the peak of the sine wave was constant, and quantified by the amplitude difference index ADI=0.5, where ADI= (Peak amplitude-Base amplitude)/ (Peak amplitude + Base amplitude). Two envelope amplitudes were chosen, and we defined high amplitude as envelope values between 0.33 for valley and 1 for peak; while low amplitude envelope was in the range 0.11 - 0.33. Finally, two sine wave phases were chosen, as stimuli could start at the valley or peak.

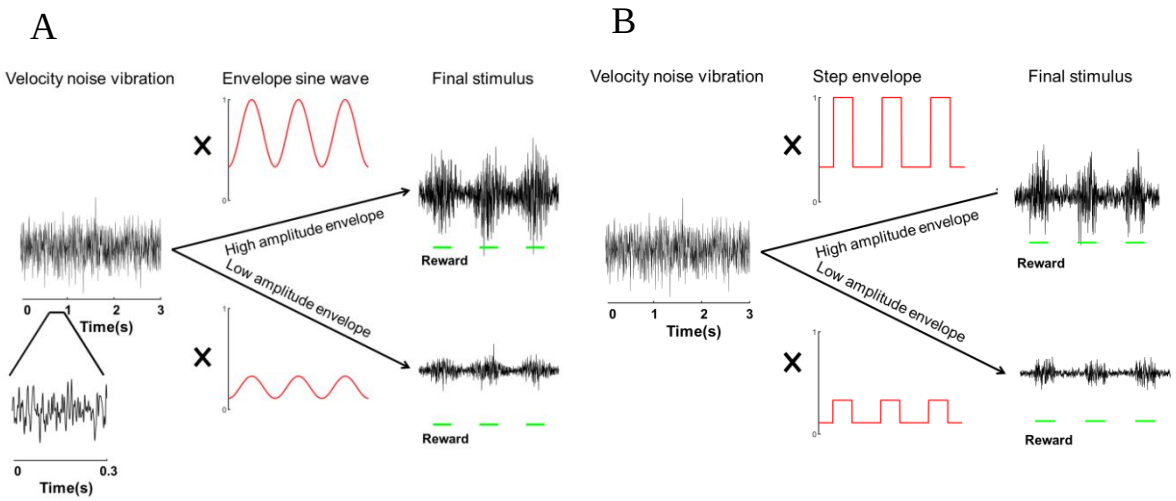

Figure 2.2: Creating the stimulus. A. A sine modulated noise vibration was obtained by multiplying a velocity noise vibration with a sine wave of different amplitudes, phases and frequencies. B. In early phases of training stimuli were modulated by a step wave, leading to two levels of amplitude.

2.4. Task

Rats were trained to detect the peak of the sine modulated noisy vibration, and respond by withdrawing from the nose-poke (Figure 2.3). On a given trial the identical stimulus was presented on whiskers on both sides of the snout, and continued while the nose-poke sensor was activated, allowing the animal to collect as much information as it chose.

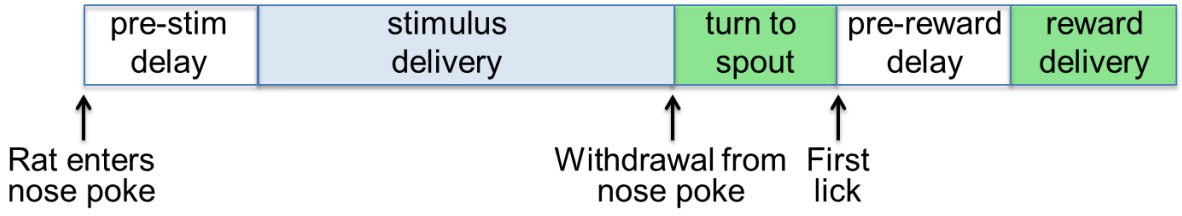

Figure 2.3: Timeline of a trial. A trial starts as the rat enters the nose poke. After a short delay the stimulus is delivered. Once the animal withdraws from the nose poke the stimulus stops and the rat has to turn to the reward spout where, if the withdrawal was correct, the water reward is delivered.

A green LED light signaled the possibility to start a new trial, and turned off at the time of the nose poke. Each trial was initiated by the rat by placing its snout in the nose-poke hole. After a short delay (randomly drawn from a Gaussian distribution with mean 250 ms and standard deviation of 50 ms) the stimulus delivery started. The stimulus was presented continuously until the animal withdrew from the nose-poke, at which time the rat received an acoustic withdrawal cue. One single spout delivered the reward after a delay from the first lick. The delay was randomly selected from a uniform distribution between 100 and 150 ms, and the reward delivery was accompanied by a reward sound. The rewarded time window covered 40% of each cycle, and was centered on the peak. To discourage very early responses, the reward for the first cycle was 0 for stimuli starting at the peak and 50% of the total reward if the stimuli started at the valley. The next trial could be initiated immediately (100 ms inter trial interval), but for some rats a larger delay (5 seconds) was imposed after incorrect trials. Animals performed on average 270 trials in each session.

Different parameters of the envelope sine wave were used to ensure that rats paid attention to the stimulus in each trial and did not use alternative strategies (for instance, fixed, stimulus-independent waiting time) for solving the task. Stimuli could have two levels of amplitude ensuring that rats did not perform the task by responding when stimuli reached a fixed amplitude threshold. The peak of the low amplitude stimuli was as intense as the valley of the high amplitude stimuli, so no universal threshold could be applied for performing the task. Moreover, the use of different frequencies and sine phases caused the rewarded periods to occur at different times in different trials, discouraging a strategy where animals could time their actions and respond after a fixed time interval.

2.5. Rat training procedure

Before training, animals were habituated to the experimenter through a 30 minutes handling session per day for 5 days. The first training step consisted of learning to activate the nose poke for a water reward. The time rats were required to spend in the nose-poke was gradually increased, but there was no cue indicating the moment at which a response could be made. Stimuli were introduced when rats could wait for more than one second in the nose poke.

First step-modulated noisy vibrations were delivered (Figure 2.2B). In this case, the velocity noise vibration was multiplied with a vector with only 2 amplitude levels. Rats had to withdraw in the high states of the stimulus to receive water. Once they performed above chance on the step-modulated stimuli (>60% correct) the sine-modulated stimuli were introduced (Figure 2.2A).

2.6. Surgery

For chronic surgeries Isoflurane (1.5-2.5%) anesthesia was delivered through a snout mask. In preparation for the surgery the animal's fur was shaved with a razor and its head was fixed in the Narashige stereotaxic apparatus. The animal was placed on a heated pad, and its temperature was constantly monitored with a thermometer inserted in the anal opening. Epigel ophthalmic moisturizing ointment was applied to prevent drying of the eyes, and lidocaine gel was used as a local anesthetic on the skin preceding the incision.

First, the skin on top of the animal's skull was cut, and the connective tissue was removed. Next, 3 screws were inserted in the bone, in contact with dura mater. These screws have a double role of fixing the implant and connecting to the reference electrode. A craniotomy was drilled according to known coordinates of premotor cortex (center +2AP, ±1.3ML from Bregma) (Erlich et al., 2011). After removing the skull covering the craniotomy, dura mater was also removed using a bent needle. Once exposed, the brain was constantly washed with phosphate buffer solution (PBS).

In order to avoid dimpling of the brain, a small drop of Vaseline-based ointment was placed in the middle of the opening, and bio compatible glue was applied on the edges of the craniotomy. Electrodes were slowly lowered in the brain, while gradually wrapping the ground/ reference wires around the screws fixed in the skull. The presence of neurons was monitored online using a TDT recording system. Once the desired depth was reached (800-1200 mm), the craniotomy was covered with silicone, and dental cement (Secure Starter Kit, Sun Medical) was used to cover the area where the skull was exposed.

Rymadil (5mg/ kg) analgesic was injected intramuscularly one hour after the anesthesia onset, and at the end of surgery. An antibiotic (Baytril, 5 mg/ kg)

was injected subcutaneously before the animal was awoken, and also delivered through the water bottle in the 48 hours following the surgery.

After the surgery rats had a week of recovery time during which water and soft food were available *ad libitum*.

At the end of the experiment rats were sedated with Urethane (1.5 mg/ kg) and transcardially perfused with 0.1% phosphate buffer solution followed by 4% paraformaldehyde. The brain was then removed and placed in paraformaldehyde at 4°C for 24 to 48 hours, and then transferred to a sucrose solution (15% to 30%). Finally, the brain was sliced with a 25 µm thickness using a microtome and stained with Nissl solution.

2.7. Electrophysiological recordings

Rats were implanted with Tucker-Davis Zif-Clip based 32 microwire arrays. Recorded digital signals were passed through a Tucker-Davis ACO-32 commutator to prevent wires from tangling when the rat turned around in the cage, and then a PZ-4 connection manifold. Next, the signal was transmitted through optical cables to a RZ2 BioAmp Processor.

A custom made OpenEx circuit was used to monitor the neural activity online, and save raw data for further processing. Together with the neural data, the behavioral epochs were also saved (nose poke, withdrawal, lick and motor trigger times).

2.8. Human psychophysics

Human participants were tested on a modified version of the task used for rats. They felt the stimuli on the fingertip of their left index finger, and responded by pressing a button placed in their right hand. Each subject performed 10 sessions of 48 trials each. For each session the participant was instructed to respond at the peak or at the valley of the stimulus (5 peak and 5 valley sessions, presented pseudorandomly).

The stimuli were created in the same manner as the stimuli used for rats but with different parameters inasmuch as performance would be nearly perfect with the rat parameters. We tested 3 envelope frequencies: 0.35, 0.7 and

1.4 Hz and two levels of amplitude. ADI was fixed at 0.5 and all trials started at the valley of the stimulus. Once the participants made a response, a front panel LED turned green or orange, indicating a correct or incorrect response.

2.9. Data analysis

All data analysis was performed using Matlab (Mathworks) scripts.

Spike sorting was performed offline using the UltraMegaSort 2000 algorhythm (Daniel N. Hill, Mehta, & Kleinfeld, 2011) implemented in Matlab.

Two types of trials were excluded from analysis of neural activity: first, trials in which the rat returned to the nose poke within 50 ms from withdrawal, in which case we considered the registered response to be due to the animal's shaking, and not to the intention to withdraw; second, trials in which the animal did not start licking the drinking spout within 4 seconds from withdrawal.

To calculate the correlation between stimulus amplitude and firing rate at every time point in the trial we divided each trial into 200 ms time bins and calculated the Spearman correlation coefficient between firing rate and average envelope amplitude in all bins. In order to avoid any bias caused by neurons changing firing rate preceding the withdrawal, from the firing rate of each time bin the average firing rate of the neuron for all trials in that time bin was subtracted. Only time bins where at least 10 trials were recorded and therefore used for averaging were considered for analysis. Similarly, when calculating the firing rate over the whole trial, we subtracted the average firing rate of all trials.

Phase coherence index was computed in order to check if the firing of premotor cortex neurons was phase locked to the envelope sine wave. The phase coherence index was calculated as follows:

$$PCI = \frac{\Sigma_j^N e^{iP_j}}{N}$$

Where PCI=phase coherence index, i=imaginary unit, N=number of spikes over all trials, j is the index of spikes pooled across all trials, and Pj=phase of firing of spike j (where the phase is the angle of the Hilbert transform of the sine wave). To select neurons with significant phase coherence ($p<0.01$), the obtained phase correlation indices were compared to the null

distribution of phase coherence indices given the number of trials recorded for each neuron.

Artificial neural network (ANN) analysis was performed using the Neural Network toolbox in Matlab. In all computations, a network with zero hidden layers was used. The input of the network was always the firing rate of neurons recorded in the same session at a specific time point, and the target could be the withdrawal time, or the time bin to which the firing rate belonged. Before applying ANN the inputs and targets were z scored.

In order to predict the withdrawal time from the neuronal firing rate the fitting tool was used. The network performance was calculated as the mean squared error between the target and the network output. In order to avoid a low network performance caused by a low ratio of number of inputs to number of trials, for each session and time bin only the 5 neurons with highest correlation coefficient between firing rate and withdrawal time were considered.

The fitting tool was also used for predicting which time bin the firing rate belonged to. The input of the network was the firing rate in consecutive 300 ms time bins, either starting with the start of the stimulation or leading to the withdrawal, and the output was the bin number 1, 2, or 3.

Finally, to classify if a firing rate belonged to the time bin just before the start of the stimulation or another time bin during the trial the pattern recognition tool was used. The network was trained on the classification between the time bin before stimulus start and the time bin preceding the withdrawal and then tested on the previous 2 time bins before withdrawal. The same analysis was performed training the network to discriminate between the time bin before start and the time bin just after start, and then tested on the 2 subsequent bins.

Network test performance was always calculated by using the leave-one-out cross validation method. For each session ANN was applied a number of times equal to the number of trials in that session. Each time the network was trained with all trials but one, and tested on the left out trial. Test performance was computed by calculating the performance (mean square error or cross entropy) given the targets and the outputs of the test trials. The expected network performance in the absence of any information from neuronal firing rate was 1, which is the standard deviation of the z scored input.

When network performance was calculated for correct and incorrect trials separately the same number of trials was considered for the 2 groups. To do so, for every session a number of trials equal to the number of incorrect trials were selected randomly from the correct trials.

3. Results

3.1. Rat behavior

Five rats were trained to detect the peak of sine modulated noisy vibrations received through their whiskers, and four of them achieved above chance performance for 10 or more consecutive sessions. The following behavioral analyses are performed on 10 sessions recorded from each rat once it reached stable performance (Figure 3.1). Trained rats learned to align their withdrawals to the peak of the stimulus, and responded mostly in the first or second envelope cycle (Figure 3.2, Figure 3.4A). The mean performance (percentage rewarded responses) of each rat was 54.5, 57.1, 70.7 and 50.6 for the 4 rats AD1, AD2, AD4 and AD5, all significantly higher than chance. Theoretical chance level was 40%, corresponding to the percentage of each cycle that was rewarded. Chance levels were also computed for each rat separately, by shuffling the animal's response times with respect to the stimulus parameters. The values of these calculated chance levels were 40.1, 41.2, 40.6 and respectively 41.4 for the 4 rats.

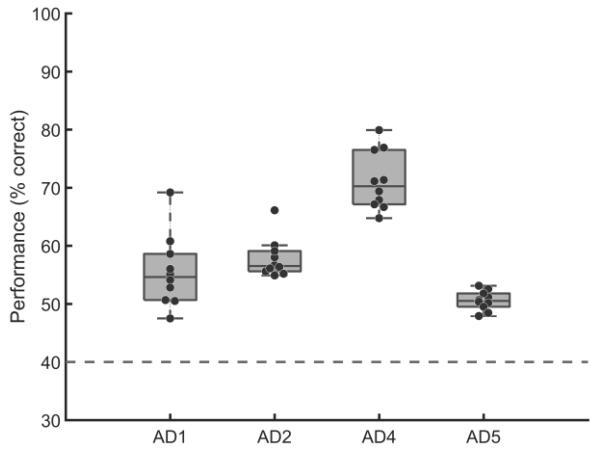

Figure 3.1: Performance (% rewarded responses) in all sessions of all rats. Each dot represents one session, the central mark of the box plot indicates the median performance value, the edges are the 25th and the 75th percentile, and the whiskers extend to the most extreme values. The grey dotted line corresponds to the theoretical chance level.

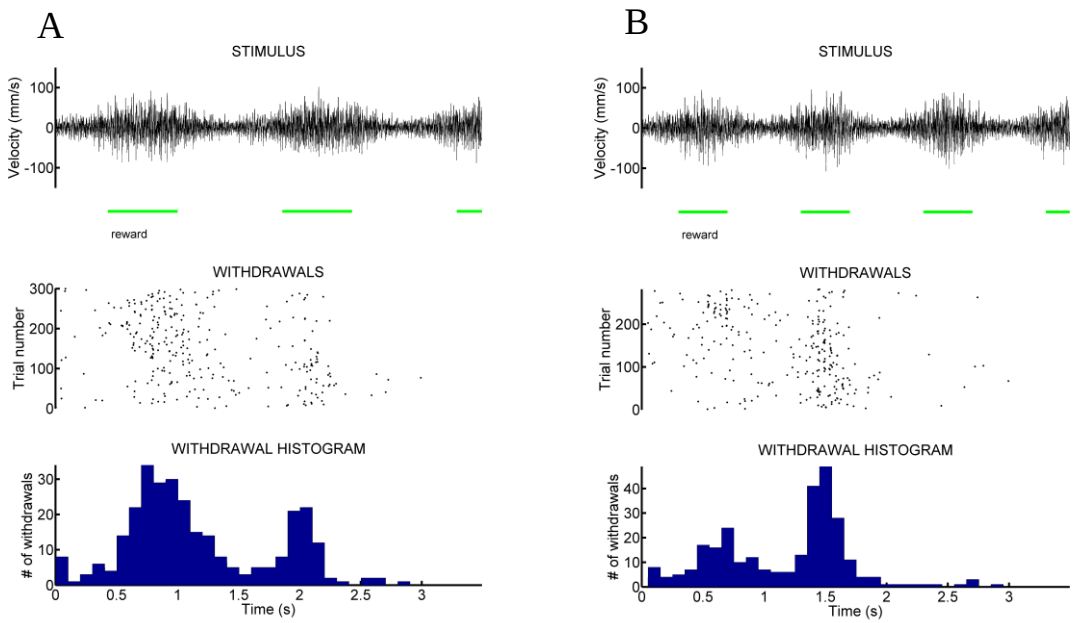

Figure 3.2: Examples of withdrawals for one rat (AD4) to all trials of frequency 0.7 Hz (A) and 1 Hz (B) starting at the valley. The green bars represent the intervals in which withdrawal would be rewarded.

Rat behavior depended on the parameters of the envelope sine wave characterizing each trial. Most importantly, rats showed better performance in trials where the higher amplitude envelope was delivered (48.5% vs 62.9%, $p<0.01$, Welch t-test on ranks) (Figure 3.3), while the envelope frequency and phase did not influence performance.

Stimulus parameters also had an effect on how long rats were willing to wait, and therefore on the cycle in which they made their responses. Rats responded in later cycles in trials characterized by low amplitude and high frequency ($p<0.01$, Welch t-test on ranks) (Figure 3.4A), suggesting that these trials required them to accumulate more evidence before responding. However, there was no significant difference between the absolute waiting times in 0.7 versus 1 Hz trials (Figure 3.4B). The absence of effect of envelope frequency and phase on performance, but their effect on the number of the response cycle indicates that rats adjusted their waiting times to achieve a satisfactory performance.

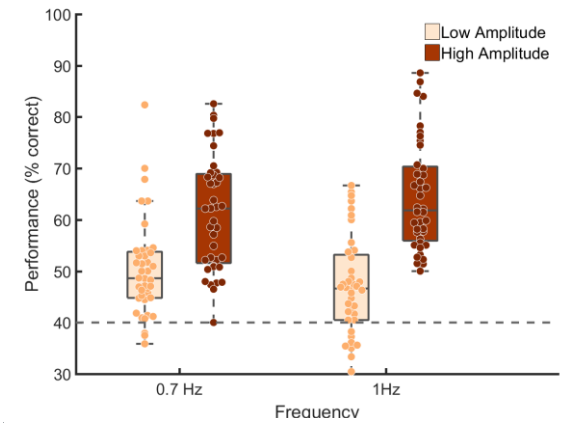

Figure 3.3: Performance of all rats in trials of different amplitudes and frequencies. Each dot represents one session. The grey dotted line corresponds to the theoretical chance level (40%).

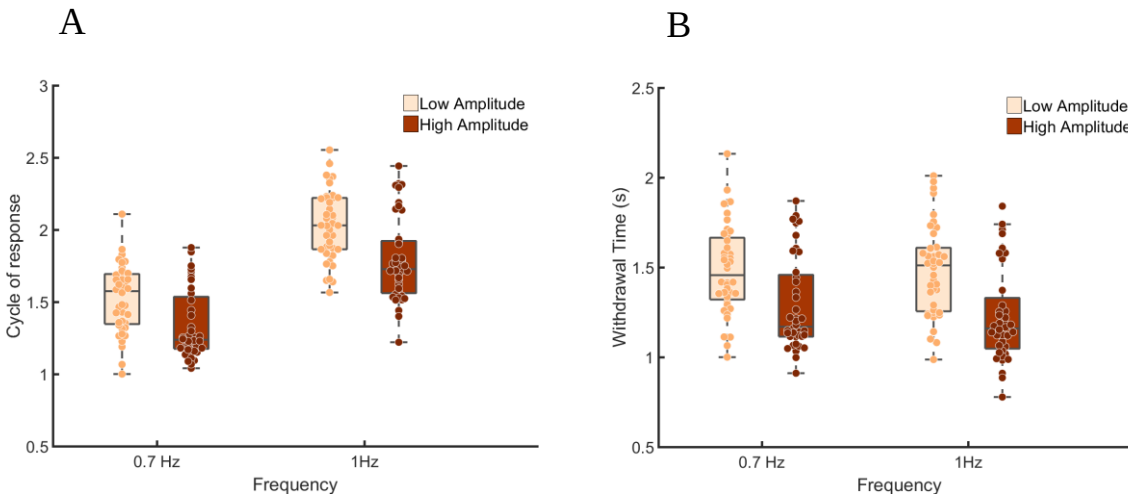

Figure 3.4: Stimulus parameters effects on withdrawal time. A. Mean number of the cycle in which the withdrawal was made. B. The average withdrawal time. Data from the 4 rats are shown, each dot representing one session.

To test whether responding in later cycles benefitted performance, we compared withdrawals made in the first and second cycles in all trials starting at the valley. Our results show that withdrawals made in the second cycle were closer to the peak than those made in the first cycle (Figure 3.5). The mean withdrawal time, where 0 is the time of the nearest peak, was 165.16 for the first cycle and -30.876 ms for the second cycle for 1 Hz stimuli and 224.99 and respectively -202.04 for 0.7 Hz stimuli. Moreover, for the 1 Hz stimuli, the standard deviation of response time to the second cycle was significantly lower

(222.5 vs 241.2 ms, $p<0.01$, t-test on bootstrapped standard deviations), showing that withdrawals were better aligned to the peak of the stimulus in trials where rats responded in the second cycle.

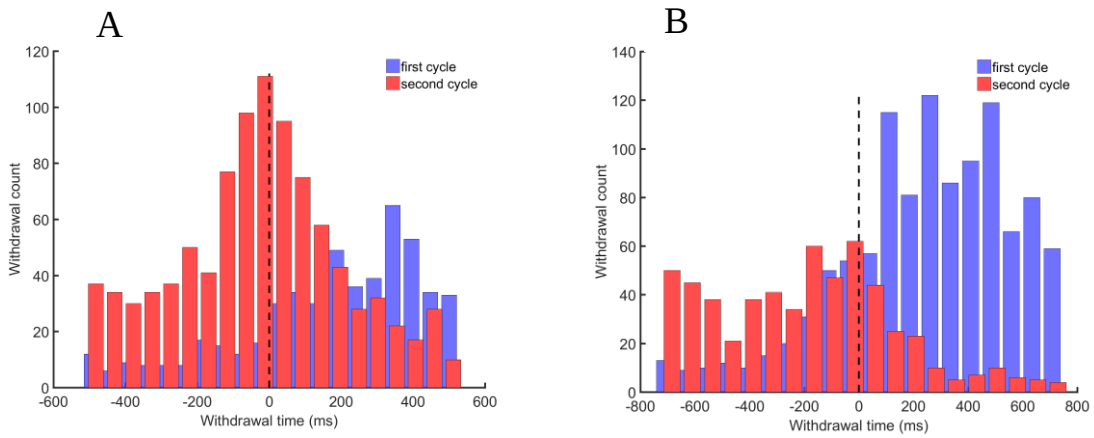

Figure 3.5: Histogram of withdrawals made in the first (blue) and second (red) cycle for all rats in trials starting at the valley. 0 on the x axis represents the time of the envelope sine wave peak. (A) frequency 1 Hz. (B) frequency 0.7 Hz.

3.2. Human psychophysics

Human participants (total 14: 6F, 8M) performed the behavioral task and achieved performances better than chance both in sessions where they were instructed to press the button at the peak and in sessions when they had to respond at the valley.

Overall performance was not significantly different for peak and valley sessions (mean performance 87.0 on peak vs 84.7 on valley sessions, $p= 0.1678$, Welch t-test on ranks). However, participants responded in later cycles in valley trials (mean cycle of response 5.78 vs 5.06; $p= 0.0016$), indicating that these trials required more evidence accumulation for making a decision. Similar to rat behavior, in both peak and valley trials humans performed better and responded in earlier cycles in high amplitude trials (Figure 3.7). Moreover, humans waited for more cycles and had lower performance in higher frequency trials, showing that it was more difficult to time their decisions when the stimulus amplitude was changing at a fast rate.

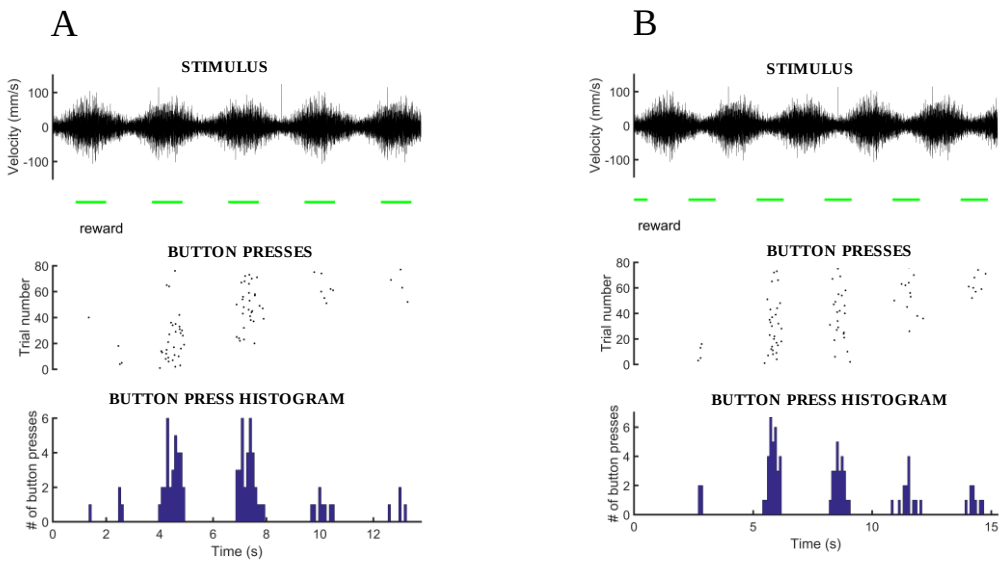

Figure 3.6: Behavior of one human subject (S1) to all trials where the instruction was to press the button at the peak (A) or valley (B) of the stimulus in all trials where the envelope frequency was 0.35 Hz. The green bars represent the intervals in which withdrawal would be rewarded.

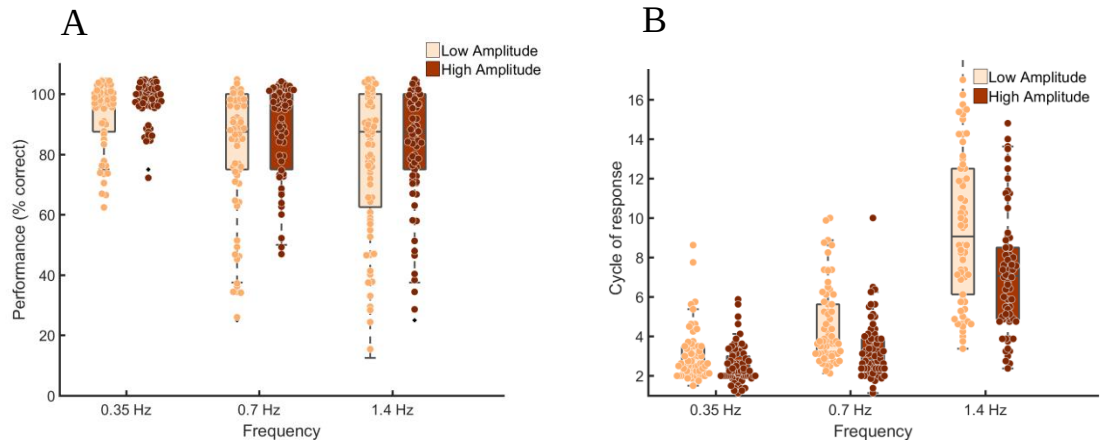

Figure 3.7: (A) Mean performance in all sessions when humans were instructed to respond at the peak of the stimulus. For visualization purposes, each performance point was shuffled by adding a value drawn randomly from a uniform distribution between -5 and 5. (B) Mean cycle of button press for all humans.

3.4. Neurophysiology

A total of 214 neurons were recorded in 34 sessions from premotor area (Figure 3.8) of 2 rats (AD2 and AD4) performing the task.

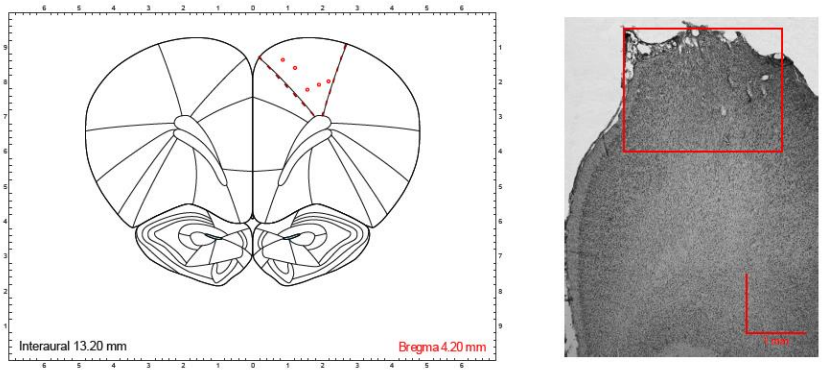

Figure 3.8: Coronal section of the brain of one of the recorded rats (AD2) at 4.2 mm anterior from Bregma. The location of the electrode tips are shown with red dots.

Premotor cortical neurons exhibited heterogeneous response patterns, with firing rates changing at different times in the trial, such as the nose poke time (Figure 3.9A), at withdrawal (Figure 3.9B), or just after withdrawal (Figure 3.9C). A large proportion (40.2%) of the recorded neurons increased their firing rate after the first lick in correct versus incorrect trials, showing participation in the reward-related network (Figure 3.10).

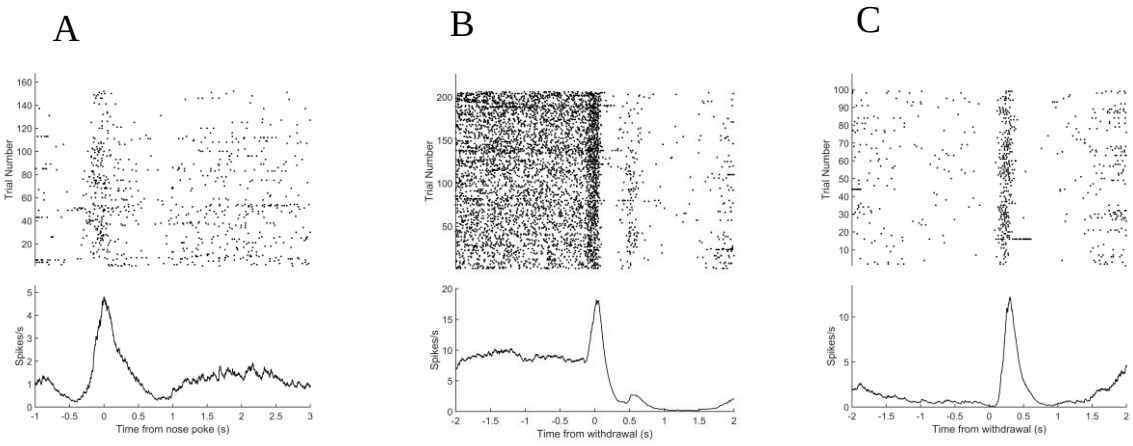

Figure 3.9: Raster plot (upper plots) and peristimlus time histogram (PSTH, lower plots) of spikes recorded from example neurons in the premotor cortex. 0 on the x axis corresponds to the nose poke (A) or withdrawal time (B and C).

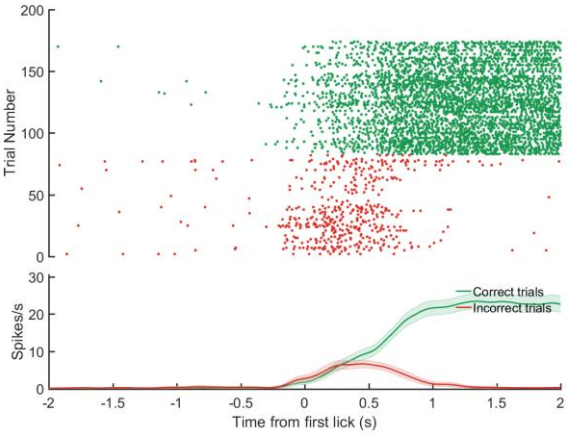

Figure 3.10: Firing rate of an example neuron with reward related activity. Trials are divided in correct (green) and incorrect (red). 0 on the x axis corresponds to the time of the first lick.

Do premotor neurons reflect the stimulus properties?

First we investigated whether premotor neurons carried information about the trial amplitude (high versus low amplitude). We calculated the Spearman correlation between the average firing rate during the whole trial and the trial amplitude and found that 23.5% of all neurons exhibited significant correlation between whole trial firing rate and envelope amplitude, 54% of which were positive (Figure 3.11A). Figure 3.11B shows the firing rates of an example neuron with positive correlation between firing rate and trial amplitude.

Furthermore, we calculated the percentage of neurons whose firing rate depended on the stimulus amplitude in correct and incorrect trials, to investigate whether the amplitude information carried by premotor cortex neurons is necessary for successfully solving the task. 13% of neurons in correct trials and 11.6% of neurons in incorrect trials showed significant correlation between the average firing rate and the overall stimulus amplitude. The amplitude of the trial is similarly decoded in correct and incorrect trials, indicating that error trials are not caused by incorrectly identifying the trial amplitude.

Next we checked whether the firing rate of premotor neurons increased in response to increased stimulus velocity, following the phases of the envelope, similar to barrel cortex neurons (Arabzadeh, Petersen, & Diamond,

2003; Antopolskiy et al. (in preparation)). For this analysis we divided each trial in 200 ms time bins and computed the Spearman correlation coefficient between the firing rate and the stimulus envelope amplitude for each bin, which produced time bins characterized by different average amplitudes for each frequency-phase-amplitude combination. 31.2% of neurons showed significant correlation between firing rate and stimulus amplitude (p=0.01), 55.2% of which were positively correlated. To exclude the possibility that the observed correlation was caused by neurons responding differently to the overall trial amplitude, we computed the same point-by-point correlation dividing trials by the envelope amplitude. Calculating the correlation for high amplitude trials we found that 13.5% of all neurons had significant correlation (62.1% positive correlation). When only low amplitude trials were analyzed only 4.65% of all neurons showed significant correlation, 80% of which were positively correlated. Better amplitude coding in high amplitude trials could be related to the observed better amplitude in high amplitude trials.

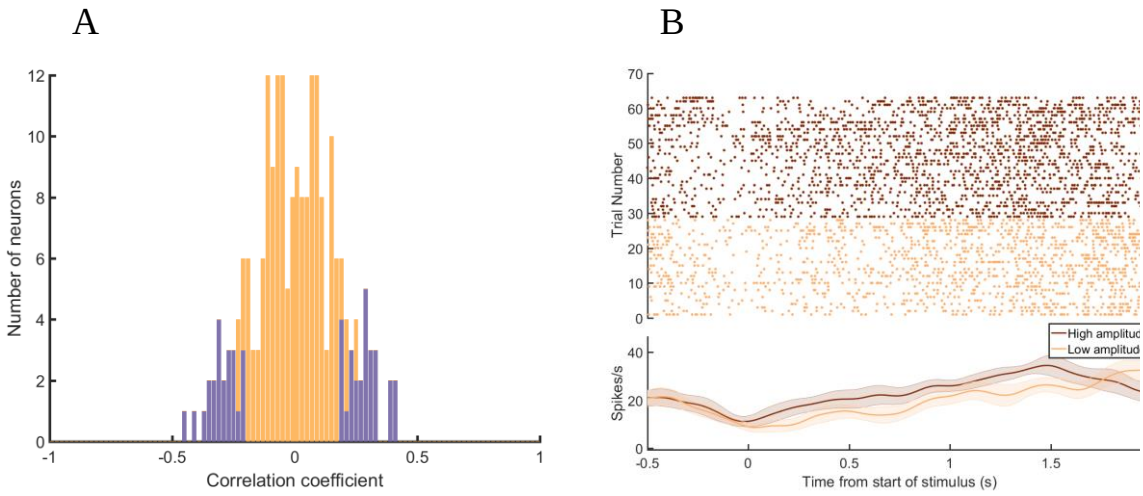

Figure 3.11: Histogram of Spearman correlation coefficients between average firing rate on each trial and amplitude identity (high/ low) (A). Firing rates of an example neuron in trials with high (dark brown) and low (light brown) amplitude (B).

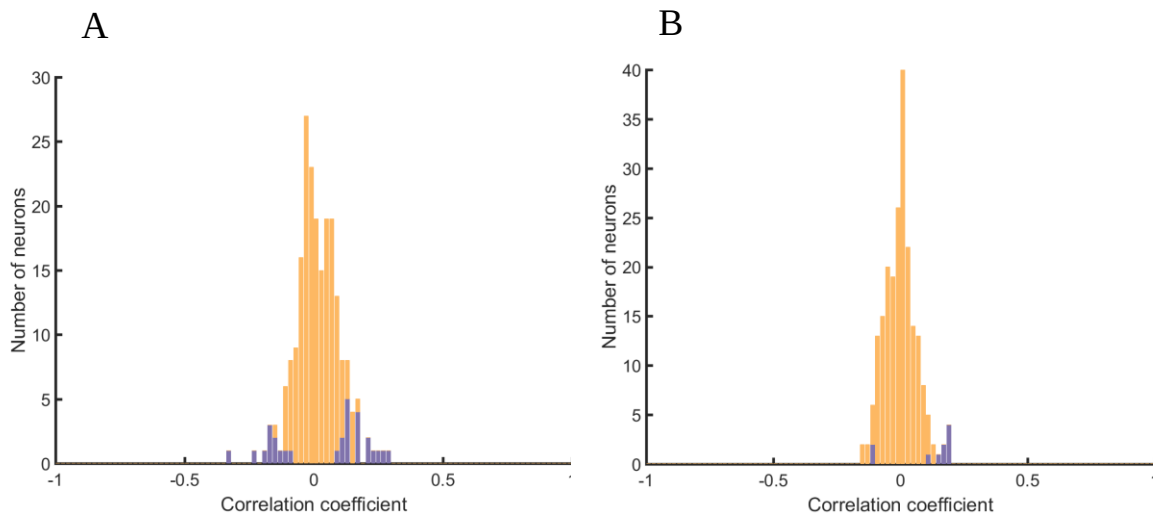

Figure 3.12: Histogram of Spearman correlation coefficients between firing rate and envelope amplitude for each neuron in high amplitude (A) and low amplitude (B) trials. Bins colored in purple are neurons with significant correlation ($p<0.01$).

In order to investigate if these results were due to neurons coding for the local amplitude or the phase of the stimulus we analyzed whether firing of premotor cortex neurons was locked to the phase of the envelope sine wave. Our results showed that 8.5% of all recorded neurons showed significant phase index. Firing of premotor cortex neurons was not only locked to the peak or the valley of the stimulus; instead it spanned the whole length of the sine wave, more strongly around the valley (Figure 3.13).

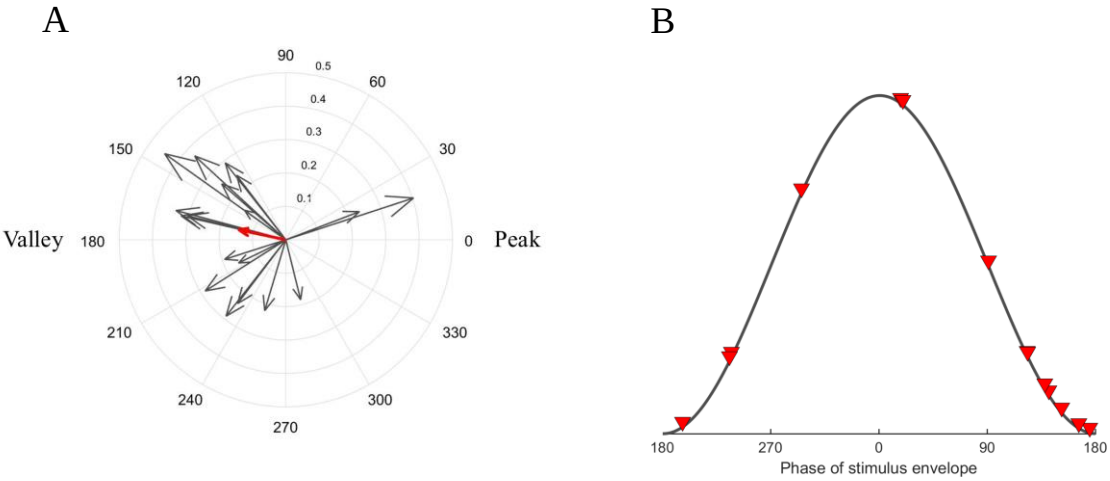

Figure 3.13: Phase coding of premotor cortex neurons. A. Polar plot of all neurons with significant phase coherence (grey) and average vector of all significant neurons (red). B. Phase locking of all significant neurons to the stimulus envelope (red triangles). The grey line represents the envelope amplitude (all stimulus amplitudes, frequencies and phases collapsed).

Therefore, the firing of premotor cortex neurons depended on the incoming sensory input at multiple levels. Many neurons carried information regarding the overall stimulus amplitude of the trial, possibly helping rats set a low or high amplitude threshold. A smaller, but significant proportion of neurons reflected the perceived stimulus amplitude at every time point in the trial. This could represent the value the rat compares to the threshold for deciding when to act. Furthermore, some neurons were phase locked to the envelope sine wave, more in the decreasing and increasing portions of the stimulus around the valley. This might be indicative of rats using the slope of the stimulus to decide when to act.

Do premotor neurons represent the passage of time?

Next we explored whether the firing rate of premotor cortex neurons predicted the withdrawal time in the trial. We aligned the neural activity by the start of the stimulus or by the animal's withdrawal and calculated the Spearman correlation between firing rate and withdrawal time (calculated as the time passed from the start of the stimulation) in 300 ms time bins. Figure 3.14 shows the percentage of neurons with significant correlation ($p<0.01$) for each time bin. The number of significant neurons is higher than the chance-expectation of 1% in all time bins, even before the start of the stimulation. More neurons show significant correlation after the stimulus starts, and as many as 26.4% of all neurons show significant correlation between 600 and 300 ms before withdrawal.

Therefore, premotor cortex neurons carry information about how much time the rat is planning to wait, evident from neurons correlated with withdrawal time when spikes were aligned with the start of the stimulation, even in the time period preceding the stimulus initiation. The firing rate of a great percentage of neurons was correlated with withdrawal time also when neuronal activity was aligned with the animal's action, therefore representing how much time has passed since the start of the stimulation. More neurons carried timing information once the stimulus started, indicating that the time at which the rat is intending to respond is updated by the perception of the stimulus.

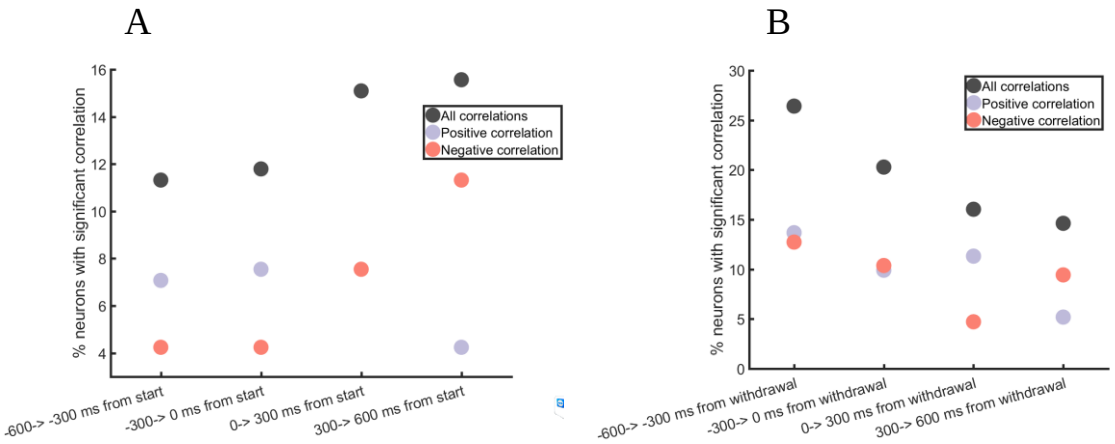

Figure 3.14: Percentage of neurons with significant Spearman correlation between firing rate and waiting time in 300 ms time bins aligned by stimulus start (A) or animal's action (B).

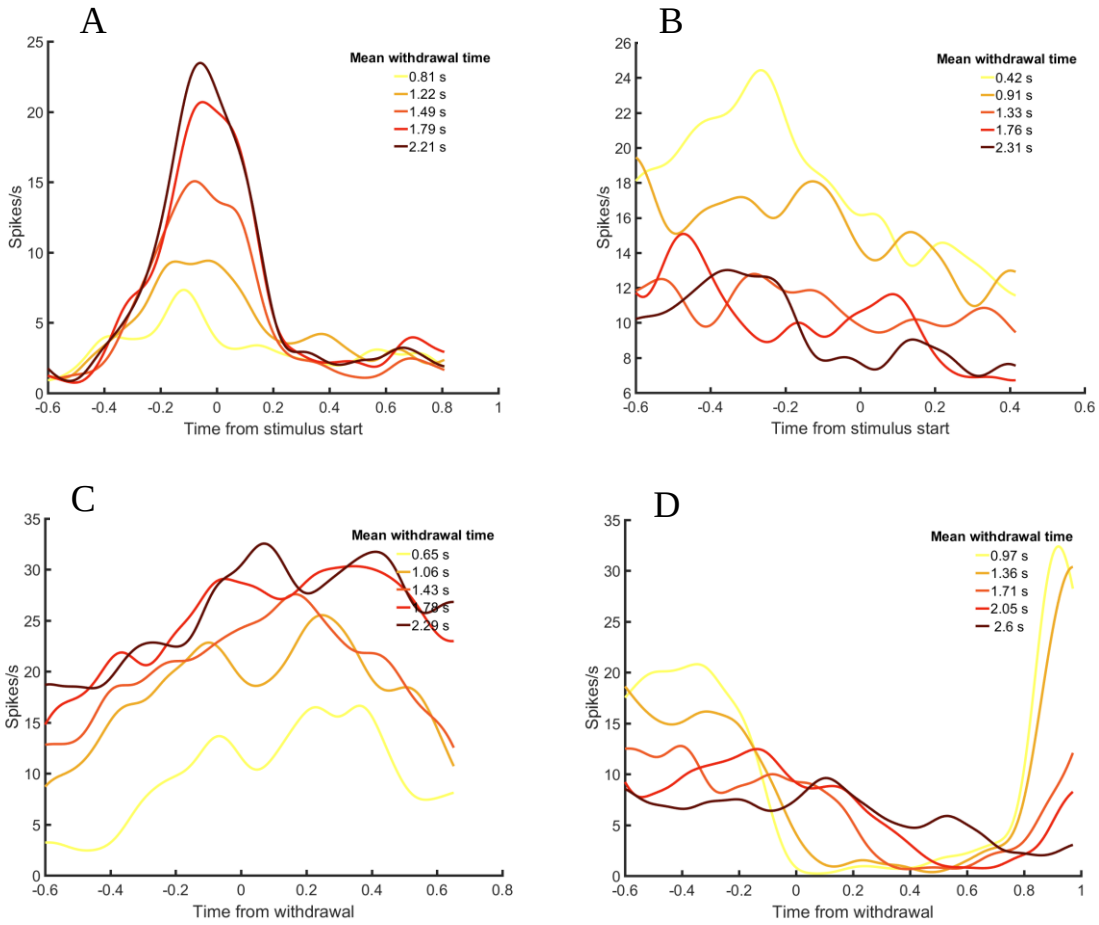

Figure 3.15: PSTH of example neurons with significant correlation between firing rate and withdrawal time. Trials were divided according to the waiting time into 5 equipopulated groups. The PSTH for each group is shown in different colors, from yellow for the shortest to dark red for the longest average withdrawal time.

We further applied an artificial neural network (ANN) to predict the withdrawal time. Inputs to the ANN were the firing rates of neurons recorded simultaneously. For each recorded session we considered only the neurons with highest correlation coefficient between firing rate and withdrawal time, and trials with withdrawal time longer than 0.6 seconds. We aligned the neural activity by the start of the stimulus or withdrawal time and applied ANN having as inputs the firing rate from four 300 ms time bins, two before the alignment point and two after.

Network performance was calculated as mean squared error between the actual waiting time and the ANN output for each test trial. Since the ANN inputs were z scored, the expected performance in the absence of any firing rate information was 1.

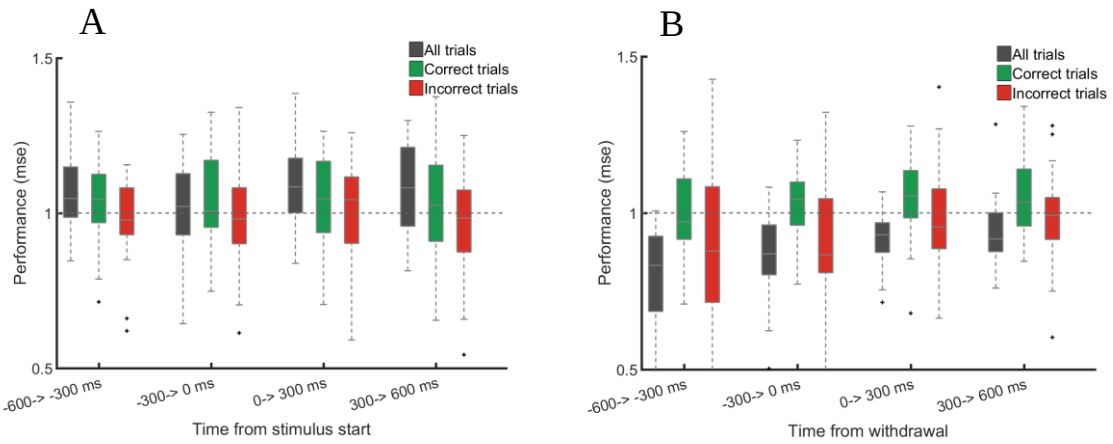

Figure 3.16: ANN performance in all sessions in 4 time bins when neural activity was aligned by start of the stimulus (A) or animal's withdrawal (B). ANN performance was obtained taking the 5 neurons with highest Spearman correlation between withdrawal time and firing rate for each bin. Trials were further divided into correct (green) and incorrect (red).

When considering the network performance in all sessions recorded from the 2 rats we observed that when spikes were aligned with the stimulus start, the average network performance was close to 1, although there are some sessions with performance lower than 1 even before the stimulus starts (Figure 3.16A). Consistent with our finding that a large number of premotor cortex neurons were correlated with withdrawal time when spikes were aligned with the animal's action, network performance was also significantly below 1, more

noticeably in the 2 time bins preceding the withdrawal. Interestingly, network performance was better in incorrect than in correct trials (Figure 3.16B). This shows that in incorrect trials rats relied on timing, while in correct trials animals relied on the stimulus and did not have to remember how much time had passed since the start of the stimulation.

Finally, we investigated whether the firing rate of premotor cortex neurons predicted the time point in the trial. In order to do so, we first divided each trial into 300 ms time bins and calculated the Spearman correlation coefficient between firing rate and the bin number for all trials together. When aligning neural activity with the start of the stimulation we obtained that 61.4% of neurons had significant correlation ($p<0.01$ Figure 3.17A) between firing rate and bin number, 54.55% of which had positive correlation. Likewise, 76.28% of all neurons had significant correlation between firing rate and bin number when neural activity was aligned by the animal's action (Figure 3.17B), 49.39% of whom had positive correlation. No difference was observed when comparing correct and incorrect trials.

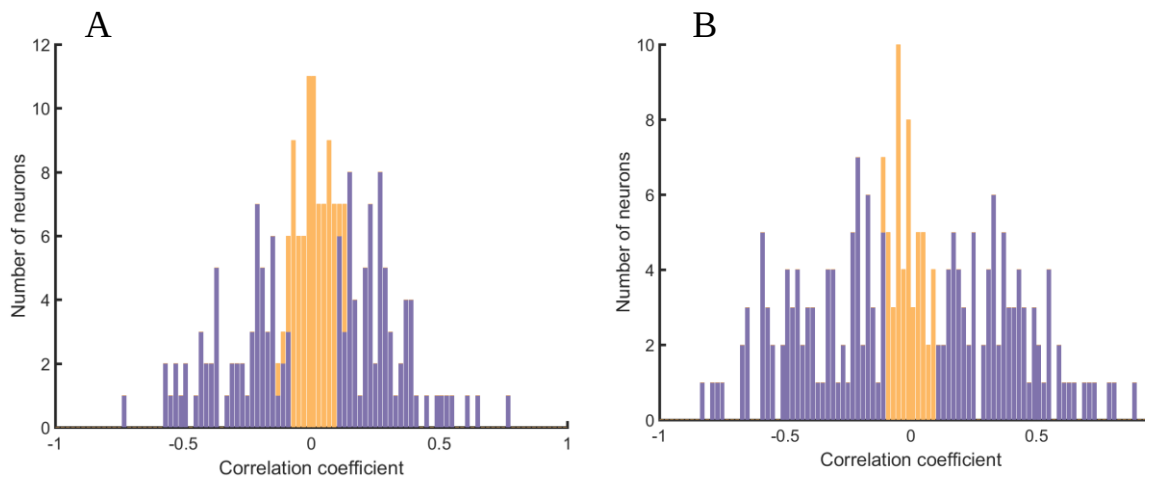

Figure 3.17: Histogram of Spearman correlation coefficients between time bin and firing rate for each neuron when neural activity was aligned by stimulus start (A) or animal's withdrawal (B). In purple the neurons with significant correlation ($p=0.01$) are shown.

Next we used the ANN to predict the current moment in the trial from neural firing rate. We chose trials with waiting time larger than 900 ms, aligned them by start of the stimulus, and divided them into 300 ms bins. The input of the network was the neuronal firing rate in each bin, and the output was the

number of the bin the firing rates belonged to (1, 2 or 3). Figure 3.18A shows the distribution of network performances for each session of recordings in the 2 rats. In most sessions network performance was lower than the expected value of 1 ($p<0.01$, one way t-test), showing that firing rate carried information about how much time has passed since the start of the stimulus. The same analysis was performed by aligning neural activity with the withdrawal time and considering the 3 time bins preceding the animal's action, again obtaining performance below 1 for most sessions (Figure 3.18B). These results show that the firing rate of premotor neurons is also informative of how much time will pass until the animal's response.

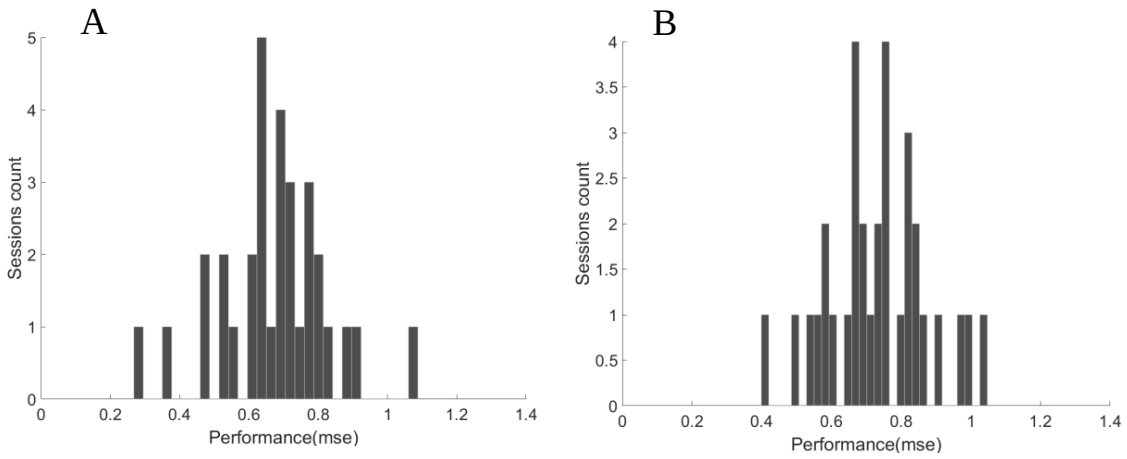

Figure 3.18: Histogram of network performance in predicting the time bin each firing rate belonged to when neural activity was aligned with start of the stimulus (A) or animal's withdrawal (B)

Therefore the firing rate of neurons in the premotor cortex throughout the trial is informative of both time passed since stimulus start and time missing until withdrawal.

Do premotor cortex neurons represent the withdrawal?

To check whether premotor cortex neurons changed their activity in prediction of the withdrawal we compared for each neuron the firing rates in the two 300 ms time bins preceding the animal's action, by computing the Welch t-test on ranks. Our results show that 35.7% of all neurons had a significant difference between the 2 time bins preceding the withdrawal (57.9% increased firing rate). This change in neural activity just before withdrawal

could be due to action prediction or stimulus coding, given that most responses rats made were during high amplitude stimulation. To disentangle between these 2 possibilities, we compared the firing rate just before withdrawal in correct and incorrect trials. Of all neurons with different firing rate in the last 2 time bins preceding the withdrawal, only 1.3% had a significant difference between correct and incorrect trials in the last bin, showing that the observed effect is not dependent on the stimulus amplitude. Figure 3.19 shows the average firing rate and the average stimulus amplitude in correct and incorrect trials of two example neurons.

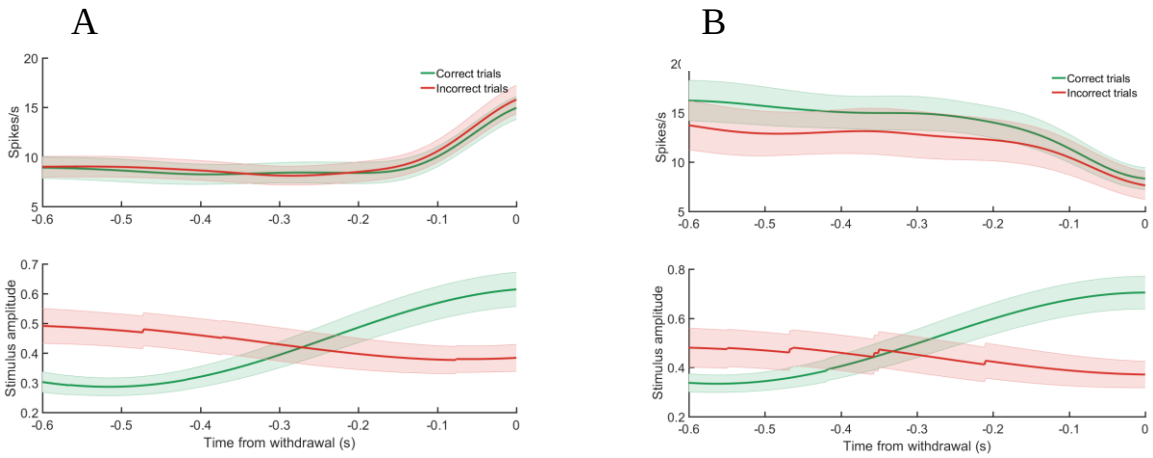

Figure 3.19: Average firing rate and the corresponding stimulus amplitude in correct versus incorrect trials, when activity is aligned with the animal's withdrawal. Two example neurons are shown: one with transient activation (A) and one whose firing rate decreases before withdrawal (B).

Finally, we used the ANN to assess how the networks of premotor neurons predicted the imminence of the animal's action in the time bin preceding the withdrawal. We trained the ANN to distinguish between the 300 ms time bin preceding withdrawal and the 300 ms time bin just before the stimulus onset, using firing rate as input (Figure 3.20B). We further tested the network on discriminating between the time bin before stimulus start and the time bin between 600 and 300 ms before withdrawal, and the time bin between 900 and 600 ms before withdrawal (Figure 3.20B). The percentage of time bins correctly classified was very high (91.0%) when discriminating between the time just before start and just before withdrawal and decreased gradually to 82.8 and then 76.6% as bins further from the withdrawal were considered

(Figure 3.20D). This result shows that as the rat approached the time of the action, neurons in the premotor cortex also approached a state most distinguishable from the state before the stimulus started.

Results were less conclusive when the network was trained to classify between firing rates belonging to the time bin before start and the time bin after start of the stimulus, and later tested on the two subsequent time bins after stimulus start (Figure 3.20A). The percentage of trials correctly classified was 77.8, 80.8 and 79.9% for the 3 time bins after withdrawal (Figure 3.20C).

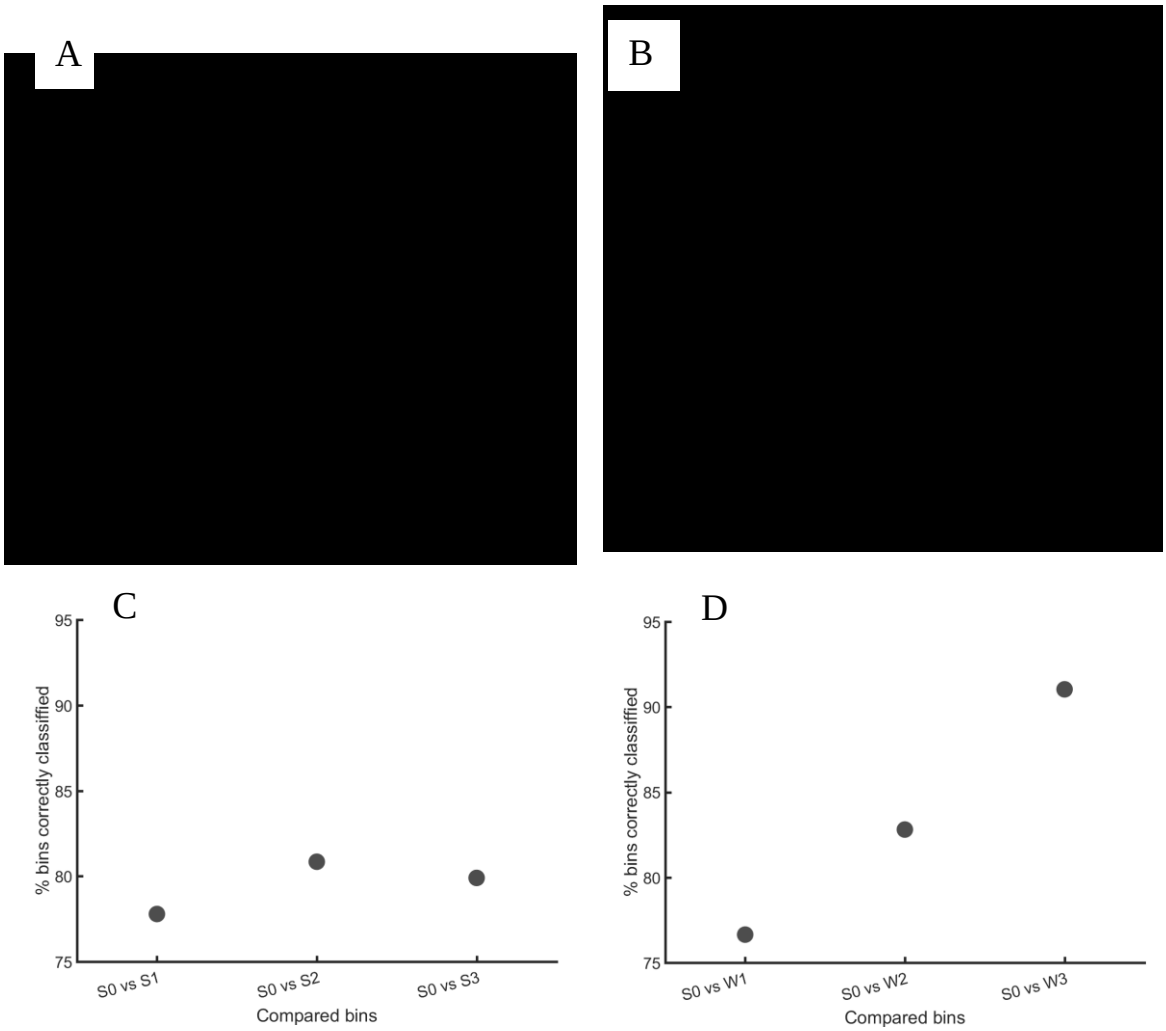

Figure 3.20: ANN was used to classify to which time bin the firing rate belonged. A, C ANN pattern recognition tool was used to classify between the 300 ms time bin before stimulus start and the time bin just after the stimulus started and then tested on the consequent 2 time bins. B, D ANN was used to classify between the time bin before stimulus start and the bin before withdrawal and tested on the 2 previous time bins. ANN was applied on every recorded session of the 2 rats and the average percentage of bins correctly classified was calculated for each time bin.

4. Discussion

We developed a new behavioral task that required subjects to integrate an input stream of sensory stimulation in order to decide for the optimal moment to initiate a motor act. This paradigm is unusual, as only a few studies have investigated how upcoming stimulus timing can be predicted based on the pattern of previous stimuli (Arnal et al., 2014; Bengtsson et al., 2009; Saleh, Reimer, Penn, Ojakangas, & Hatsopoulos, 2010), but none incorporated the preparation of a well-timed response. Our study is innovative because subjects are required to time their motor response precisely depending on the incoming stimulus. Moreover, we implemented this paradigm in humans and rats, which allowed us to compare the behavioral strategies in the two species.

4.1. Rats and humans employ similar behavioral strategies

In our task rats and humans were presented with noisy vibrations (Fassihi, Akrami, Esmaeili, & Diamond, 2014, Antopolsky et al. (in preparation)), modulated by an envelope sine wave that made them periodically increase and decrease in amplitude. The subjects' task was to respond at the peak of the stimulus. By changing the amplitude, frequency and stimulus onset phase of the envelope sine wave the subjects were discouraged from using alternative strategies to solve the task, such as applying an amplitude threshold or timing their responses relative to trial onset.

Rats learned to solve the task by withdrawing at the peak amplitude, and did so better than chance. Rats withdrew at different times according to stimulus parameters, indicating that they did not employ a rigid timing strategy for solving the task. This claim is further supported by the fact that chance levels calculated by shuffling the waiting times with respect to the stimulus parameters is approaching the theoretical chance level of 40%, corresponding to how many trials would be rewarded if rat withdrew randomly throughout the trial. Moreover, performance was better than chance for both levels of amplitude, indicating that rats did not solve the task by simply applying an amplitude threshold irrespective of the perceived stimulus.

Humans spent more time than rats identifying the stimulus peak and reached a performance that was considerably higher. This was probably due to differences in patience and goals in the two species - rats were trying to get the largest reward in the shortest time, while humans were trying to perform the task correctly. However, there were many similarities in the behavioral strategies employed by rats and humans, such as responding in later cycles in low amplitude trials as well as having lower performance in those trials. This indicates that these trials were more difficult and necessitated more evidence integration. Moreover, although human performance was similar in trials where they were instructed to button press at the peak and trials where they were required to respond at the valley of the stimulus, they responded in later cycles for valley trials, indicating that timing responses to the valley of the stimulus was more difficult.

At the beginning of each trial, subjects were not aware of the current stimulus parameters, so they were required to accumulate stimulus evidence in order to decide when to initiate the motor act. Rats have previously proven to be capable of accumulating evidence in time (Douglas et al., 2006; Reinagel et al., 2012). A valid strategy for solving the task would be to identify the amplitude and the frequency of the envelope and withdraw as soon as enough evidence has been accumulated. A minimum necessary for a high performance would be to gather evidence in the first cycle of the stimulation and withdraw during the second cycle. Withdrawals rats made in the second cycle were better aligned to the stimulus peak, indicating that gathering stimulus information during the first cycle improved prediction of the peak in the second cycle. Also, both rats and humans responded in later cycles in low amplitude and high frequency trials, indicating that these trials required more evidence accumulation before making a decision.

4.2. Rat premotor cortex neurons carry task-relevant signals

We subsequently investigated if neurons in the premotor cortex carried information about the stimulus or withdrawal time. Our choice of the brain area was based on previous studies demonstrating the role of primate and rat premotor cortex in decision making (J I Gold, Shadlen, J.I., & M.N., 2000), time

processing (Rao, Mayer, & Harrington, 2001), and stimulus perception (R Romo et al., 1993). Finally, human frontal areas have been shown to be involved in predicting the moment at which a stimulus will occur based on the pattern of previous incoming stimuli (Arnal et al., 2014; Saleh et al., 2010; Schubotz & von Cramon, 2002).

First, we investigated if the rat premotor cortex neurons carried information regarding the incoming stimulus for each trial.

A high percentage of premotor cortex neurons showed correlation between firing rate over the whole trial and the amplitude identity (high/ low) for each trial. Amplitude information could be helping rats solve the task by identifying the maximum amplitude in each trial and withdrawing when the maximum amplitude was perceived again.

Information about the value of the stimulus at each point during the trial was rather low in premotor cortex neurons, in contrast to barrel cortex neurons that follow the course of the stimulus, firing more when the stimulus amplitude increases (Antopolsky et al. (in preparation)). Nevertheless, information about the stimulus intensity was present in the premotor cortex, particularly in high amplitude trials, which could explain a better performance in these trials. Moreover, neurons showed little phase coherence with the envelope sine wave, and were mostly phase locked to the increasing and decreasing amplitude sub-segments around the valley of the envelope sine wave, possibly indicating a strategy where rats take into account the slope of the stimulus for deciding when to act.

Interestingly, we found a correlation between the neuronal firing rate and the waiting time at different times in the trial, even before the start of the stimulation, indicating that neurons carried a signal related to the rats' willingness to wait. A larger than expected percentage of neurons had firing rates correlated with the withdrawal time both when neural activity was aligned with the start of the stimulus, indicating how much time will pass until the rat makes its response, and when spikes were aligned with the animal's withdrawal, which is associated with the amount of time having passed since the start of the stimulus. These neurons resemble the transiently active neurons found by Murakami et al (Murakami et al., 2014). The information regarding the withdrawal time increased at the start of the stimulation and was highest in the time bins preceding the withdrawal, possibly showing that the waiting time

signal was updated by stimulus information. Performance of an artificial neural network in predicting the withdrawal time was higher in incorrect than in correct trials, revealing that on incorrect trials rats relied on timing, while in correct trials they paid attention to the stimulus and did not have to keep in mind how much time had passed since the start of the stimulation.

Neural firing in the premotor cortex also predicted the imminence of the withdrawal in the time period preceding the rat's response, as expected from previous studies (Crutcher & Alexander, 1990; Riehle & Requin, 1989; Tanji et al., 1980). This is shown by a significant difference between firing rates in the 2 time bins before withdrawal. Moreover, the network of premotor cortex neurons was evolving towards a state of better discriminability throughout the trial, starting from the last time bins just preceding the start of the stimulation.

4.3. Implications of the present work

The data set collected thus far has not yet been exhaustively exploited. It can therefore be used to address additional questions about timing decisions in the rat premotor cortex. For example, although we showed that the firing rate of premotor cortex neurons is correlated with waiting time, it would be valuable to explore whether this time related signal influences the process of acquiring stimulus evidence in the aforementioned area. Moreover, in order to further our understanding of timing based decisions in the rat brain it would be beneficial to build a model that takes into account the timing and stimulus related activity of premotor cortex neurons before and during the start of the stimulation in order to predict the animal's withdrawal time.

Furthermore, the present study allows us to speculate about the implications of other brain areas in timing decisions in response to sensory stimuli. If we expect the barrel coretex (Antopolsky et al. (in preparation)) neurons to encode stimuli by increasing their firing rate in response to higher intensity of vibrations, we predict that other high order association areas would carry signals related to the animal's decision. For example, primate lateral intraparietal neurons have been shown to integrate weak, slowly arriving sensory information to generate a decision (Churchland, Kiani, & Shadlen, 2008; Roitman & Shadlen, 2002; M. N. Shadlen & Newsome, 1996; N. N. Shadlen & Newsome, 2001). We expect rat posterior parietal cortex neurons to

also carry signals related to the accumulation of noisy evidence. Moreover, the rat prefrontal cortex has been shown to be crucial in working memory tasks (Yang, Shi, Wang, Peng, & Li, 2014). We expect this area to also participate in the task by representing different working memory aspects of the task, such as the current trial amplitude. Both of these areas may also carry timing signals relative to the task (Dietrich & Allen, 1998; Leon, Leon, Shadlen, & Shadlen, 2003; Xu, Zhang, Dan, & Poo, 2014)

4.4. Conclusion

Our results show that rats combined two behavioral strategies for solving the task. The first one is to observe the incoming stimulus and withdraw once enough stimulus evidence was accumulated. That the rats benefitted from accumulating more evidence about the stimulus is evident from better-timed responses to the stimulus peak when withdrawals were made in the second cycle of stimulation. More precisely, our results suggest that rats identified the overall amplitude of the trial and withdrew once the perceived stimulus amplitude reached a certain threshold. This hypothesis is supported by the fact that many neurons fired differently depending on the trial overall amplitude. Furthermore, rats performed better in high amplitude trials where the absolute difference between peak and valley was higher and therefore setting a threshold was less challenging. Premotor cortex neurons also carried some information about the instantaneous amplitude of the stimulus, which could be the value rats compared to the reference threshold to decide when to withdraw. This information was better represented in correct trials, indicating that it may be used by rats for solving the task. The second strategy rats could engage in was to ignore the stimulus and simply time their responses. A small percentage of premotor cortex neurons had firing rates correlated with the withdrawal time even before the stimulus was presented, indicating that the premotor cortex contains a “patience” variable that maybe shaped by but is independent from the accumulated evidence variable. Throughout the trial the waiting time was better predicted from the firing rate in incorrect than correct trials, showing that in incorrect trials animals kept track of time.

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